National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1566b

Oyster Tissue

This Standard Reference Material (SRM) is intended primarily for use in evaluating analytical methods and instruments used for the determination of the concentrations of selected elements and proximates, selected fatty acids, total dietary fiber, as well as the caloric content in marine bivalve tissue, foods, or similar materials. A unit of SRM 1566b contains approximately 25 g of freeze-dried oyster tissue.

Certified Concentrations of Constituent Elements: Certified values for 22 elements and for methylmercury are provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been fully investigated or accounted for by NIST. Certified values are based on results obtained by a single primary method with confirmation by other methods, or with two or more critically evaluated independent methods [1]. The certified value for sulfur is the result of a single NIST method. All other certified values are weighted means of results obtained using two or more independent methods.

Reference Concentration Values: Reference values are noncertified values that are the best estimates of the true values; however, the values do not meet NIST criteria for certification. Such values are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. Reference values for elemental concentrations are provided in Table 2. Reference values for proximates, nitrogen, total dietary fiber, selected fatty acids, and caloric content are provided in Table 3.

Information Concentration Values: Information concentration values for selected fatty acids are provided in Table 4. These are noncertified values with no reported uncertainties as there is insufficient information to assess uncertainties. The information values are given to provide additional characterization of the material, and are not recommended for use to monitor or assess analytical performance.

Expiration of Certification: The certification of this SRM lot is valid until **01 June 2010**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is nullified if the SRM is contaminated or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of certification. If substantive changes occur that affect the certification prior to the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

The coordination of the technical measurements leading to the certification of this SRM was performed by R.R. Greenberg of the NIST Analytical Chemistry Division. Coordination of technical measurements of organic compounds and methylmercury leading to certified, reference, and information values was performed by K.E. Sharpless and S.A. Wise of the NIST Analytical Chemistry Division.

The NIST analysts and cooperating laboratories that participated in the characterization of this SRM are listed in Appendix A.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert.

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Certificate Issue Date: 17 January 2001

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Preparation of the oyster tissue material was performed by B.J. Porter of the NIST Analytical Chemistry Division and M.P. Cronise, C.N. Fales, and D.G. Friend of the NIST Standard Reference Materials Program.

The statistical analysis of the data was performed by J.H. Yen, L.M. Gill, and M.S. Levenson of the NIST Statistical Engineering Division.

NOTICE AND WARNING TO USERS

Storage: The material should be kept in its original bottle, tightly closed, and stored in a desiccator over magnesium perchlorate $Mg(ClO_4)_2$, at temperatures between 10 °C to 30 °C. It should **NOT** be exposed to intense sources of radiation, including ultraviolet light or sunlight.

Use: A minimum sample mass of 250 mg of material is necessary for any certified value in Table 1 to be valid within the stated uncertainty. This amount of material should be on a dry-mass basis (see Instructions for Drying). The contents of the bottle should be shaken well before each use, closed tightly **immediately** after use, and stored as described above.

Instructions for Drying: Prior to removal of subsamples for elemental analysis, the contents of the bottle should be thoroughly mixed. Before the mass determination, samples of SRM 1566b must be dried to constant mass by one of the following procedures:

1. Drying at room temperature for *at least* 5 d over $Mg(ClO_4)_2$ in a desiccator.
2. Vacuum drying at room temperature for *at least* 24 h at a pressure of approximately 30 Pa (0.2 mm Hg) using a cold trap.
3. Freeze drying for *at least* 5 d at a pressure of approximately 30 Pa (0.2 mm Hg).

The analyst should ascertain that the material has indeed reached constant mass. Although the above procedures have been generally sufficient, in a few instances the time needed to reach constant mass was longer than listed above. If the constituents of interest are volatile, a separate subsample of the oyster tissue should be removed from the bottle at the time of analysis and dried to determine the concentration on a dry-mass basis.

SOURCE, PREPARATION, AND ANALYSIS[1]

Source and Preparation of Material: The oysters (*Crassostrea virginica*, an American Eastern oyster) used for the preparation of SRM 1566b were purchased from Bon Secour Fisheries, Inc., Bon Secour, AL. The oysters were collected from the Gulf of Mexico, shucked, rinsed twice to remove sediment and shells, packed and sealed in polyethylene bags, and frozen. The frozen oysters and fluids were shipped in styrofoam coolers containing dry ice to NIST. At NIST, the oysters and fluids were ground in a Robot-Coupe Vertical Cutter Mixer that was equipped with a stainless steel bowl and titanium blades. The oyster tissue was blended for 100 s into a slurry; approximately 5 kg of slurry was poured into each of 40 specially cleaned aluminum trays outfitted with temperature probes, and frozen at -20 °C. The trays were taken to a large freeze-drying facility at the Frederick Cancer Research and Development Center, Natural Products Group in Frederick, MD. The freeze-dryer's initial temperature was -45 °C and gradually increased to a temperature of 10 °C over a period of five days. The freeze-dried material was stored at -20 °C, then broken into smaller pieces, blended in the Robot-Coupe Mixer, jet milled, and homogenized in a V-blender for 30 min to 40 min. The material was radiation sterilized (^{60}Co) at Neutron Products, Inc., Dickerson, MD, for approximately 5 h at 3 Mrad and then aliquoted into amber bottles.

Description of Calculations Used in Value Assignment

A. Certified Values and Their Uncertainties

Sulfur

The certified value of sulfur is the result of a single NIST method, thermal ionization mass spectrometry (TIMS), with confirmation by a second NIST method. Its uncertainty is expressed as an expanded uncertainty, U, and is calculated as $U = ku_c$. The quantity, u_c, is the combined standard uncertainty calculated according to the ISO Guide [2] that accounts for the combined components of uncertainty for the method at one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to five degrees of freedom and a 95 % prediction interval.

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[1]Certain commercial equipment, instrumentation, or materials are identified in this certificate to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Methylmercury

The results for methylmercury are expressed as mg/kg mercury. The certified value is the mean of results from four different laboratory analyses of SRM 1566b using four different analytical methods. The expanded uncertainty in the certified value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the ISO Guide [2] and k is the coverage factor. The value, u_c, is intended to represent, at the level of one standard deviation, the combined effect of all the uncertainties in the certified value. Here, u_c is given by the standard error of the mean of the four analyses. The coverage factor, k, is determined from the Student's t-distribution corresponding to three degrees of freedom and 95 % confidence for each analyte.

All Other Elements

All other certified values are weighted means of results from two or more analytical methods. For these certified values, the uncertainty is calculated as $U = ku_c + B$. The quantity, u_c, is the combined standard uncertainty calculated according to the ISO Guide [2], which accounts for the combined effect of the within variance for all methods at one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each element. The term, B, is a bias adjustment for the difference between methods which is the maximum difference between the certified value and the method means [3]. Because of heterogeneity, the uncertainty associated with calcium and thorium takes the form of a prediction interval.

Table 1. Certified Concentration Values of SRM 1566b[1]

Element	Mass Fraction (%)			Element	Mass Fraction (%)		
Calcium[c,g]	0.0838	±	0.0020	Potassium[g,i,k]	0.652	±	0.009
Chlorine[g,i]	0.514	±	0.010	Sodium[b,g]	0.3297	±	0.0053
Magnesium[c,g]	0.1085	±	0.0023	Sulfur[d,i]	0.6887	±	0.0140

Element	Mass Fraction (mg/kg)			Element	Mass Fraction (mg/kg)		
Aluminum[g,l]	197.2	±	6.0	Mercury[a,n] (total)	0.0371	±	0.0013
Arsenic[g,j]	7.65	±	0.65	Methylmercury[o,p,q,r] (as mercury)	0.0132	±	0.0007
Cadmium[e,h]	2.48	±	0.08	Nickel[e,j,k]	1.04	±	0.09
Cobalt[e,g]	0.371	±	0.009	Rubidium[e,g]	3.262	±	0.145
Copper[e,g,h]	71.6	±	1.6	Selenium[g,j]	2.06	±	0.15
Iron[g,k,m]	205.8	±	6.8	Silver[e,g]	0.666	±	0.009
Lead[c,e]	0.308	±	0.009	Thorium[c,g]	0.0367	±	0.0043
Manganese[e,g]	18.5	±	0.2	Vanadium[e,g]	0.577	±	0.023
				Zinc[g,k]	1 424	±	46

[1] Dry-mass basis

[2] Analytical Methods:

  [a] Cold vapor atomic absorption spectrometry at NIST
  [b] Flame atomic emission spectrometry at NIST
  [c] Isotope dilution inductively coupled plasma mass spectrometry at NIST
  [d] Isotope dilution thermal ionization mass spectrometry at NIST
  [e] Inductively coupled plasma mass spectrometry at NIST
  [f] Inductively coupled plasma optical emission spectrometry at NIST
  [g] Neutron activation analysis (instrumental) at NIST
  [h] Neutron activation analysis (radiochemical) at NIST
  [i] Prompt gamma activation analysis at NIST
  [j] Electrothermal atomic absorption spectrometry at National Research Council of Canada (NRCC)
  [k] Isotope dilution inductively coupled plasma mass spectrometry at NRCC
  [l] Inductively coupled plasma mass spectrometry at NRCC
  [m] Inductively coupled plasma optical emission spectrometry at NRCC
  [n] Neutron activation analysis (radiochemical) at Jožef Stefan Institute, Ljubljana, Slovenia
  [o] Gas chromatography with atomic emission detection at NIST
  [p] Gas chromatography with atomic fluorescence detection at Jožef Stefan Institute, Ljubljana, Slovenia
  [q] Cold vapor atomic absorption spectrometry at Jožef Stefan Institute, Ljubljana, Slovenia
  [r] Cold vapor atomic absorption spectrometry at Research Centre Jülich, Jülich, Germany

Table 2. Reference Concentration Values of SRM 1566b[1]

These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification; therefore unrecognized bias may exist for some measurands in this matrix.

Element	Mass Fraction (%)		
Nitrogen[a]	7.6	±	0.4

Element	Mass Fraction (mg/kg)			Element	Mass Fraction (mg/kg)		
Antimony[b]	0.011	±	0.002	Strontium[c]	6.8	±	0.2
Barium[d]	8.6	±	0.3	Tin[d]	0.031	±	0.008
Boron[a]	4.5	±	1.9	Uranium[d]	0.2550	±	0.0014
Hydrogen[a]	7.2	±	0.4				

[1] Dry-mass basis

[2] Analytical Methods:

[a] Prompt gamma activation analysis at NIST
[b] Neutron activation analysis (instrumental) at NIST
[c] Inductively coupled plasma mass spectrometry at NIST
[d] Isotope dilution inductively coupled plasma mass spectrometry at NRCC, Ottawa, Canada

Table 3. Reference Concentration Values of Proximates, Nitrogen, Total Dietary Fiber, Selected Fatty Acids (as Triglycerides) and Calories in SRM 1566b

These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification; therefore unrecognized bias may exist for some measurands in this matrix.

Constituent	Mass Fraction as received (%)[a]			Mass Fraction dry-mass basis (%)[a]		
Moisture	4.6	±	3.6	0 (by definition)		
Solids	95.4	±	3.6	100 (by definition)		
Carbohydrates	43.4	±	3.2	45.4	±	1.7
Ash	3.87	±	0.09	4.05	±	0.15
Fat	5.5	±	1.2	5.8	±	1.1
Protein[b]	42.6	±	1.3	44.7	±	2.6
Protein Nitrogen[b]	6.82	±	0.20	7.16	±	0.42
Total Dietary Fiber	6.5	±	1.6	6.8	±	1.4
Caloric Content[c]	(394	±	20) kcal/100 g	(412.7	±	5.9) kcal/100 g
Tetradecanoic Acid (C14:0) (Myristic Acid)	0.403	±	0.075	0.421	±	0.057
Pentadecanoic Acid (C15:0)	0.079	±	0.016	0.083	±	0.013
Hexadecanoic Acid (C16:0) (Palmitic Acid)	2.16	±	0.20	2.27	±	0.15
(Z)-9-Hexadecenoic Acid (C16:1) (Palmitoleic Acid)	0.362±		0.063	0.064	0.379	±
Heptadecanoic Acid (C17:0) (Margaric Acid)	0.168	±	0.022	0.178	±	0.023
Octadecanoic Acid (C18:0) (Stearic Acid)	0.424	±	0.054	0.444	±	0.041
(Z)-9-Octadecenoic Acid (C18:1) (Oleic Acid)	0.86 ±		0.20	0.90±	0.21	
(all-Z)-5,8,11,14, 17-Eicosapentaenoic Acid (C20:5) (Timnodonic Acid)	0.065	±	0.012	0.068	±	0.014

[a] Each reference value, expressed as a mass fraction of the material on an as-received or dry-mass basis, for the above measurands is an equally weighted mean of results from the cooperating laboratories listed in Appendix A. Each of four laboratories analyzed one portion from each of three bottles of SRM 1566b using their routine methods. Determinations were performed on the material "as received," with conversion of results to a dry mass basis using moisture values determined by the four laboratories on separate subsamples taken from each of the three bottles. The uncertainty in the reference values is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO Guide [2]. The expanded uncertainty is calculated as, $U = ku_c$, where, u_c, is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Table 5.

[b] The protein concentration was calculated from the nitrogen values reported by the laboratories using a conversion factor of 6.25. The value for protein is the mean of the individual protein calculations. If the mean nitrogen values above are used for calculation, the mean protein concentrations are 42.6 % and 44.7 % on a wet mass and dry mass basis, respectively.

[c] The value for caloric content is the mean of the individual caloric calculations. If the mean proximate values are used for calculation, with caloric equivalents of 9, 4, and 4 for fat, protein, and carbohydrate, respectively, the mean caloric content is 394 kcal/100 g and 412.7 kcal/100 g on an as-received and dry-mass basis, respectively.

Table 4. Information Concentration Values of Selected Fatty Acids (as Triglycerides)

These concentrations are provided as information values only because the disagreement among the results was greater than expected for reference values or because results were reported by a limited number of laboratories. The data for these information values are not of sufficient quality or quantity to adequately assign uncertainties.

Fatty Acid	Mass Fraction, as received (%)[a]	Mass Fraction, dry-mass basis (%)[b]
(E)-9-Octadecenoic Acid (C18:1) (Elaidic Acid)	0.16	0.17
(Z,Z)-9,12-Octadecadienoic Acid (C18:2) (Linoleic Acid)	0.097	0.10
(all Z)-5,8,11,14-Eicosatetraenoic Acid (C20:4) (Arachidonic Acid)	0.029	0.031
Docosanoic Acid (C22:0) (Behenic Acid)	0.017	0.018
Tetracosanoic Acid (C24:0) (Lignoceric Acid)	0.022	0.023

[a] Each information value, reported on an as-received and dry-mass basis, is the equally weighted mean of results reported by the laboratories shown in Appendix A. These values are based on results from determinations by two to four of the laboratories and are included to provide additional characterization of the material; no uncertainties are provided. Analytical methodology information is provided in Table 5.

[b] Determinations were performed on the material "as received," with conversion of results to a dry-mass basis using moisture values determined by the four laboratories on separate subsamples taken from each of three bottles.

Table 5. Analytical Methods Used by Collaborating Laboratories for the Determination of Proximates, Fatty Acids, Calories, and Total Dietary Fiber in SRM 1566b

Ash	mass loss after ignition in a muffle furnace
Calories	calculated; [(9 × fat) + (4 × protein) + (4 × carbohydrate)]
Carbohydrate	calculated; [solids – (protein + fat + ash)]
Fat	sum of individual fatty acids
Fatty acids	hydrolysis followed by gas chromatography
Moisture	mass loss after drying in a vacuum oven (3 laboratories) or forced-air oven (1 laboratory)
Protein nitrogen	Dumas (1 laboratory); modified Dumas (1 laboratory); Kjeldahl (2 laboratories)
Protein	calculated from nitrogen using a factor of 6.25
Solids	calculated; (sample mass – moisture)
Total dietary fiber	enzymatic gravimetry

REFERENCES

[1] May, W., Parris, R., Beck, C., Fassett, J., Greenberg, R., Guenther, F., Kramer, G., Wise, S., Gills, T., Colbert, J., Gettings, R., and MacDonald, B., "Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements," NIST Special Publication 260-136, January 2000; available at http://www.cstl.nist.gov/nist 839/srminfo.html.

[2] *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed. ISO, Geneva, Switzerland, 1993; see also Taylor, B.N. and Kuyatt, C.E., Guidelines for Evaluating and Expressing Uncertainty of NIST Measurement Results," NIST Technical Note 1297, U.S. Government Printing Office, Washington DC, (1994); available at http://physics.nist.gov/Pubs/.

[3] Schiller, S. and Eberhardt, K., "Combining Data from Independent Chemical Analysis Methods," Spectrochimica Acta, 46B, No. 12, pp. 1607-1613, (1991).

Appendix A

NIST Analysts, Analytical Chemistry Division, National Institute of Standards and Technology

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Collaborating Laboratories and Analysts for Elemental Determinations

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Collaborating Laboratories for Proximates, Fatty Acids, Total Dietary Fiber, and Calorie Determinations

Covance Laboratories, Madison, WI, USA
Lancaster Laboratories, Lancaster, PA, USA
Medallion Laboratories, Minneapolis, MN, USA
Southern Testing and Research Laboratories, Wilson, NC, USA